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CLINICAL RESEARCH

Leptin levels in the differential diagnosis between benign and malignant ascites

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Abstract

AIM: To evaluate the role of leptin levels in the differential diagnosis of ascites.

METHODS: Ascitic leptin, TNFα and serum leptin levels were measured in 77 patients with ascites (35 with malignancies, 30 cirrhosis and 12 tuberculosis). Control serum samples were obtained from 20 healthy subjects. Leptin and TNFα levels were measured by ELISA. Body mass index (BMI) and percentage of body fat (BFM) by skin fold measurement were calculated for all patients and control groups. Peritoneal biopsy, ascites cytology and cultures or biochemical values were used for the diagnosis of patients.

RESULTS: In patients with malignancies, the mean serum and ascites leptin levels and their ratios were significantly decreased compared to the other patient groups and controls. In tuberculosis peritonitis, ascitic fluid TNFα levels were significantly higher than malignant ascites and cirrhotic sterile ascites. BMI and BFM values did not distinguish between patients and controls.

CONCLUSION: In patients with malignant ascites, levels of leptin and TNFα were significantly lower than in patients with tuberculous ascites.

Key words: Leptin; Benign ascites; Malignant ascites; Tuberculosis; Cirrhosis

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INTRODUCTION

Leptin, a product of the obese gene, is a multifunctional hormone secreted predominantly by adipocytes. It was discovered in 1994 by Friedman *et al*[1]. The importance of leptin in the regulation of energy balance, food intake and body composition through a central feedback mechanism has been demonstrated in both animal and human studies[2-5]. In humans, circulating leptin levels exhibit a particularly strong positive correlation with the body fat mass (BFM), body mass index (BMI) and sex. Serum leptin concentration is higher in obese than in lean subjects and in females than in males[6]. On the other hand, leptin serum levels can be altered in various diseases[7].

Liver cirrhosis is associated with hypercatabolism and malnutrition that leads to increased energy expenditure[8]. Previous studies have shown that circulating leptin levels are modestly elevated in patients with alcoholic and posthepatic cirrhosis[9-12]. Another study reported that the ascitic fluid leptin levels of cirrhotic patients with sterile ascites are on average two times higher than circulating levels of this hormone[13]. Serum leptin levels have been reported to be reduced in cancer patients[14,15]. Abramov *et al*[16] found low leptin levels in serum, pleural, peritoneal fluids and in Meigs' syndrome as well. In the literature, there is no study about leptin levels in malignant ascites. In patients with active pulmonary tuberculosis, a previous study found increased leptin concentrations and a correlation with increased concentrations of tumor necrosis factor than control groups. After antituberculosis therapy, leptin levels of these patients were elevated, but this increase was not statistically significant[17]. However, in a recent study in patients with active pulmonary tuberculosis, leptin levels were determined to be decreased. Reduced body fat content secondary to anorexia and cachexia during the active disease period was claimed as a reason for this[18]. To our knowledge, there is no study

about this subject in either ascites or serum of patients with tuberculosis peritonitis.

There are problems in the differential diagnosis of ascites. Especially diagnosis of exudative ascites (including lymphoma, various forms of peritonitis, peritoneal carcinomatosis, and peritoneal tuberculosis) is a dilemma. Non-invasive tests such as laboratory tests, acid-fast stain and culture of the ascitic fluid, ADA levels are usually insufficient for the differential diagnosis of ascites[19,20]. Therefore laparoscopy with directed biopsy is necessary for the diagnosis of ascites. In the light of these findings of our prospective study, leptin may be accepted as a new criterion for the differential diagnosis of malignant and benign ascites.

MATERIALS AND METHODS

Materials

The study population consisted of 77 patients with ascites and 20 healthy people who were recruited from the Department of Gastroenterology, Gaziantep University Hospital, Gaziantep, Turkey. The patients were divided into three groups. Group Ⅰ consisted of 35 patients with various types of malignant diseases (seven ovarian adenocarcinoma, ten adenocarcinoma of the colon, five lymphoma, seven pancreatic adenocarcinoma, six gastric adenocarcinoma). Mean age was 56.2 ± 17.4 years (20 men and 15 women). Group Ⅱ included 30 cirrhotic patients (mean age 55.05 ± 13.26 years, 15 men and 15 women). The diagnosis of cirrhosis was based on the typical findings of hepatic cirrhotic appearance, splenomegaly, esophageal varices and ascites (by ultrasonography and upper gastrointestinal endoscopical examinations), with biochemical data. Etiology of cirrhosis was hepatitis C virus in fifteen patients, hepatitis B virus in thirteen patients and cryptogenic in two patients. The severity of cirrhosis was graded according to the Child-Pugh classification[21]. All of the patients were Child C. Group Ⅲ consisted of 12 patients with tuberculous peritonitis (these patients were diagnosed with laparoscopic peritoneal biopsy, mean age 51.9 ± 15.3 years, 5 men and 7 women). The control groups included 20 healthy volunteers (mean age 52.9 ± 13.3 years, 10 men and 10 women). Exclusion criteria were diabetes mellitus, existence of pleural effusion, gastrointestinal bleeding, spontaneous bacterial peritonitis and renal failure, treatment with corticosteroids, immunosuppressive agents and oral contraceptive agents within the last 6 mo. Control group consisted of healthy individuals with normal medical history, physical examination and blood biochemistry. None of them have had a restriction of diet for loosing weight during the last three months. The local ethics committee approved the study and written consents were received from all participants.

Methods

Blood samples were obtained in the early morning after an overnight fasting (12 h). The blood was centrifuged at 3000 rpm for 20 min at 4℃ and serums were was stored at -80℃ until analysis of leptin concentrations. Biochemical analyses were done during the same day. After paracentesis of peritoneal fluid, ascitic fluid was collected under sterile conditions and seeded in both aerobic and anaerobic cultures. It was immediately centrifuged at 3000 rpm for 20 min at 4℃ and stored at -80℃ in tubes for assessment of leptin levels. Ascitic fluid was also obtained for routine biochemical analysis, cytologic exam and white blood cell counts.

Body composition such as BMI, skin fold thickness, body fat percentage (BFP), and BFM analysis was performed in patients and controls. BMI was defined for study populations as weight divided by height squared (kg/m^2). Skinfold thickness was measured at four different sites on the left side of the body (triceps, biceps, subscapular and supra-iliac) using a Holtain skinfold caliper. Percent body fat (%BF) was calculated using the Jacksons' formula[22], BFM was calculated using BFP and body weight as in kilograms, and triceps skinfold thickness less than a 10^{th} of a percentile[23] was excluded. Serum and ascitic fluid leptin and serum TNFα levels were measured by a commercially available ELISA kit (Quantikine Human Leptin Immunoassay; RD Systems). The lower limit of detection was 0.05 μg/L. The intra-assay and interassay coefficients of variation was below 12%.

Statistical analysis

Results were given as mean ± SE. Comparisons between and among the groups were made using a non-parametric test (Mann-Whitney *U* test) and one way ANOVA variant analyses. Statistical analysis for the comparison of serum and ascitic fluid analyses was carried out by means of Wilcoxon matched pairs signed rank test. The correlation among numerical data was analyzed by the Pearson correlation coefficient (*r*). Spearman's rank correlation test was used for estimation of the level of the association between two variables. Data were statistically evaluated using the SPSS v10.0 (Statistical Program for Social Science, version 10.0, Chicago, IL, USA) software packages. $P < 0.05$ was taken as significant.

RESULTS

Patients' demographic data

The demographic, anthropometrical characteristics of all patients and control groups are showed in Table 1. There were no statistically significant differences between three patients groups and the controls regarding BMI, BFP and BFM ($P > 0.05$).

Serum and ascitic fluid leptin and TNFα levels

The main biochemical analyses, leptin, and TNFα levels of the ascitic fluid of the patients and serum leptin levels are shown in Table 2. None of the patients had spontaneous bacterial peritonitis. Serum concentrations of leptin were significantly elevated in tuberculosis and cirrhotic patients compared with serum levels in control and malignant patients groups ($P = 0.0001$). There were no statistically significant differences in serum leptin levels between cirrhosis and tuberculosis patient groups ($P > 0.05$). In addition serum leptin levels of malignant patients were

Table 1 Demographic and anthropometrical characteristics of all subjects

Characteristic	Mean age ± SD (yr)	BMI (kg/m^2)	BFP (%)	BFM (kg)
Cirrhotic patients (n = 30)	55.05 ± 13.26	22.1	26.4	17.3
Male (n = 15)	57.2 ± 12.3	21.5	23.8	16.6
Female (n = 15)	53.3 ± 13.05	22.6	28.8	17.9
Malignant ascites (n = 35)	56.2 ± 17.4	22.4	26.8	17.4
Male (n = 20)	58.2 ± 15.3	22.1	24.2	17.1
Female (n = 15)	54.1 ± 14.2	22.8	29.4	17.9
Tuberculous peritonitis (n = 12)	51.9 ± 15.3	22.6	26.9	17.6
Male (n = 5)	53.8 ± 13.2	22.2	24.6	17.2
Female (n = 7)	50.1 ± 12.4	23	29.4	18.1
Control (n = 20)	52.9 ± 13.3	22.7	27	17.7
Male (n = 10)	54.6 ± 11.8	22.5	24.8	17.3
Female (n = 10)	51.6 ± 10.9	22.9	29.2	18.2

Table 2 Ascitic leptin, TNFα and serum leptin levels in study population (mean ± SD)

	Control subjects	Cirrhotic patients	Malignant ascites	Tuberculous peritonitis
Leptin (ascites) (μg/L)	-	7.19 ± 3.43	2.45 ± 1.25	5.59 ± 1.08
Leptin (serum) (μg/L)	2.93 ± 0.32	3.77 ± 2.04	1.30 ± 0.63	3.72 ± 0.64
TNFα (ascites) (ng/L)	-	30.36 ± 6.37	29.65 ± 7.08	65.5 ± 18.4
Total proteins (ascites) (g/L)	-	15.4 ± 6.3	26.1 ± 11.5	28.2 ± 21.9
Albumin (ascites) (g/L)	-	7.1 ± 4.0	14.3 ± 7.2	13 ± 11.6
LDH (ascites) (U/L)	-	142.97 ± 93.99	546.5 ± 473.8	351.40 ± 378.82
Glucose (ascites) (mg/L)	-	1253.6 ± 348.1	1057.2 ± 443.8	1176 ± 482.3
Total leukocyte (ascites) (× 10^6/L)	-	0.43 ± 0.46	976 ± 608.4	1.16 ± 1.17
Neutrophils (ascites) (× 10^6/L)	-	0.22 ± 0.31	668.19 ± 416.3	0.44 ± 0.63
Lymphocytes (ascites) (× 10^6/L)	-	0.12 ± 0.01	223.34 ± 102.3	0.75 ± 0.32

significantly lower than control groups ($P < 0.001$, Table 2).

On the other hand, the levels of leptin in ascitic fluid were significantly higher in tuberculosis and cirrhotic patients than in patients with malignant effusions ($P < 0.001$). In cirrhotic patients, ascitic fluid levels of leptin were higher than in patients with tuberculosis peritonitis, but the difference was not statistically statistically significant ($P > 0.05$, Table 2). Leptin levels were significantly higher in ascitic fluid than in serum ($P < 0.01$ Wilcoxon test), with a mean ascites/serum ratio of approximately 2.0 in all patient groups (Table 2). Serum and ascitic fluid leptin levels showed significant positive correlation in all patient groups (Malignant group: $r = 0.979$, $P = 0.0001$; Cirrhotic group: $r = 0.999$, $P = 0.0001$; Tuberculosis group: $r = 0.995$, $P = 0.0001$).

Ascites TNFα levels were significantly increased in patients with tuberculosis peritonitis, when compared to the other patient groups. Ascites TNFα levels were not statistically significant between cirrhosis and malignant ascites groups ($P < 0.001$, Table 2). We observed that ascitic fluid TNFα levels were significantly positively correlated with ascitic leptin levels in the tuberculosis peritonitis group ($r = 0.979$, $P = 0.0001$). However, this correlation was not observed in the other patient groups.

DISCUSSION

Recently, several studies have suggested that serum levels of leptin are significantly elevated in patients with chronic liver disease. The relation between serum levels of leptin, TNFα, and associated liver fibrosis have been investigated in patients with chronic hepatitis C by Piche *et al*[24]. Serum fasting levels of leptin were significantly more elevated than the control group and a significant correlation has been demonstrated between serum levels of leptin, TNFα and severity of liver fibrosis in those patients[24]. Several studies found higher leptin levels among female alcoholic cirrhotic patients than the control group[10,11] and Henriksen *et al*[11] suggested that the elevated circulating leptin in patients with alcoholic cirrhosis was most likely caused by a combination of decreased renal extraction and increased release from fat tissue areas. However, in an animal study with chronic ethanol consumption rats, it has been shown that serum concentrations of tumor necrosis factor were increased and leptin was induced in the liver and peripheral adipose tissues by TNFα[25]. In addition, experimental and animal studies have shown that TNFα is one of the major leptin producer and regulators in anorexia and inflammation[26-28]. It has also been suggested that leptin mediates anorexia in chronic inflammatory states[29]. Anorexia and increased energy expenditure usually accompanies cirrhosis[30]. In previous studies, association between the severity of posthepatitis cirrhosis and serum leptin levels is controversial[12,30,31]. In cirrhotic patients, a significant negative correlation was found between serum levels of leptin and Child-Pugh score[31]. On the contrary, a significant elevation in leptin levels was observed as the Child-Pugh score worsened independently of gender and BMI in cirrhotic patients. Anorexia and hypermetabolism in cirrhosis are held responsible for this[12]. In our study, we also observed that circulating leptin levels were increased in the non-alcoholic cirrhosis group caused by viral hepatitis and not in the control group.

Previous studies have demonstrated that TNFα levels are elevated in the ascitic fluid of cirrhotic patients with spontaneous bacterial peritonitis[32]. Nevertheless Giannini *et al*[13] found that serum and ascitic TNFα levels are significantly elevated but serum and ascites TNFα levels were not correlated and the ascitic fluid leptin levels were either twice as high as serum levels or positively correlated in cirrhotic patients with sterile ascites. High leptin levels in ascites were explained as a result of TNFα stimulation and production of intra-abdominal leptin. The results of our study support the hypothesis of Giannini *et al*[13]. In 30 patients with active pulmonary tuberculosis in Turkey, a recent study found increased leptin concentrations and a correlation with TNFα[17]. Similar results were reported in another study from Turkey involving 25 patients with tuberculosis[33]. After antituberculosis therapy in two studies, leptin levels were more elevated. One study from

Indonesia found lower leptin concentrations in 60 HIV-negative patients with active tuberculosis compared to 30 healthy control[18]. In another study performed in with patients with tuberculous pleural effusions, it has been demonstrated that serum and pleural fluid levels of tumor necrosis factor were significantly elevated compared with transudative pleural effusion[34]. For the time, no study dealing with leptin concentrations in tuberculosis peritonitis has been reported in the literature to our knowledge. In this study, we found that ascitic fluid TNFα levels in tuberculosis peritonitis are significantly elevated when compared to the other patients groups, serum and ascitic leptin levels were significantly higher than in malignant patients. In addition, we observed that ascitic fluid TNFα levels were significantly positively correlated with ascitic leptin levels in the tuberculosis peritonitis group. Our findings support that in tuberculosis peritonitis, TNFα may be a major stimulating factor of leptin secretion.

Low serum leptin levels have been observed in patients with malignancy[14,15]. It has been shown that serum leptin levels of prostate cancer are lower than benign prostatic hyperplasia[14]. On the other hand, it has recently been demonstrated that in patients with pre-menopausal carcinoma in situ of the breast cancer, serum leptin levels were decreased compared with the control group[15]. In a case report, while levels of leptin in the serum, peritoneal and pleural fluids were lower in patient Meigs' syndrome, serum levels were increased following surgical therapy of the tumor along with the resolution of ascites and pleural fluids. Abramov and coworkers suggested that mediators of the tumoral tissue inhibit leptin secretion and excision of the tumoral tissue increased leptin levels[16]. In patients with malignant pleural effusions, levels of TNFα were significantly lower than in patients with tuberculous effusions[34]. In a recent study, we investigated the importance of ascitic fluid LDH level and LDH isoenzyme activities in patients with malignant and nonmalignant ascites. LDH level, LDH-4 activity and LDH-5 activity were found to be significantly higher, and LDH-1 activity was found to be lower in malignant ascites when compared with nonmalignant ascites. We conclude that ascitic LDH and its isoenzyme pattern may be helpful for the differential diagnosis of ascites[35].

According to our knowledge, there is no study about leptin levels in malignant ascites in the literature. In the present study, we observed that ascitic fluid TNFα levels, serum and ascitic fluid leptin levels of malignant patients are significantly lower than tuberculosis patients. Is the reason for low leptin levels that some mediators released by the tumor which inhibit leptin secretion? Or does decreased body fat, secondary to malnutrition and cachexia caused by tumor, results in decreased levels of leptin? More studies are necessary to explain these. In summary, our study has demonstrated that serum and ascitic fluid leptin levels of malignant patients are significantly lower than tuberculosis peritonitis and cirrhotic patients with sterile ascites. These findings suggest that leptin may be an important marker in the malignant ascitic fluid. Especially, leptin as a tumour marker may be useful in differential diagnosis of benign and malignant ascites.

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